

**Hypoxia-inducible factor prolyl hydroxylase 1 (PHD1) deficiency promotes
hepatic steatosis and liver-specific insulin resistance in mice**

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Supplementary Figure 1. Effects of whole-body PHD1 deficiency on tissue-specific expression of PHDs isoforms. The mRNA expression of the various HIF-PHDs isoforms (*Egln2*: PHD1; *Egln1*: PHD2; *Egln3*: PHD3; *Ldha*: LDHA) was measured by RT-qPCR in liver (A), epididymal white adipose tissue (B) and skeletal muscle (C) of WT (open bars) and PHD1^{-/-} (black bars) mice on standard chow diet. The results are expressed relative to the housekeeping gene RPLP0 as fold change vs WT mice. Data are means ± SEM (n=7 for WT; n=13 for PHD1^{-/-}). # p<0.05 vs WT mice.

Supplementary Figure 2. PHD1 deficiency promotes weight gain and insulin resistance but does not worsen high-fat diet-induced metabolic alterations. WT (open bars) and PHD1^{-/-} (black bars) mice were fed a low-fat (LFD, 10 % fat) or high-fat (HFD, 45% fat) diet for 6 weeks. Body weight was monitored throughout the experimental period (A). Delta (Δ) change in body weight from the start of diet (B), plasma triglycerides (C), total cholesterol (D), glucose (E) and insulin (F) levels were determined and HOMA-IR (G) was calculated in 6-hour unfed mice at week 6. An intraperitoneal GTT (2 g/kg of total body weight) was performed in 6-hour unfed mice at week 5. Blood glucose levels were measured at the indicated time-points (H), and the area under the curve (AUC) of the glucose excursion curve was calculated as a measure of glucose tolerance (I). The plasma insulin level during ipGTT was measured at 15 minutes (J). An intraperitoneal ITT (0.5 U/kg total body weight) was performed in 6-hour unfed mice at week 6. Blood glucose levels were measured at the indicated time-points (K) and the AUC of the glucose excursion curve was calculated as a measure of insulin resistance (L). Data are means ± SEM (n=4 for LFD-WT; n=7 for LFD-PHD1^{-/-}; n=5 for HFD-WT; n=7 for HFD-PHD1^{-/-}). * p<0.05 vs LFD-fed mice, # p<0.05 vs WT mice.

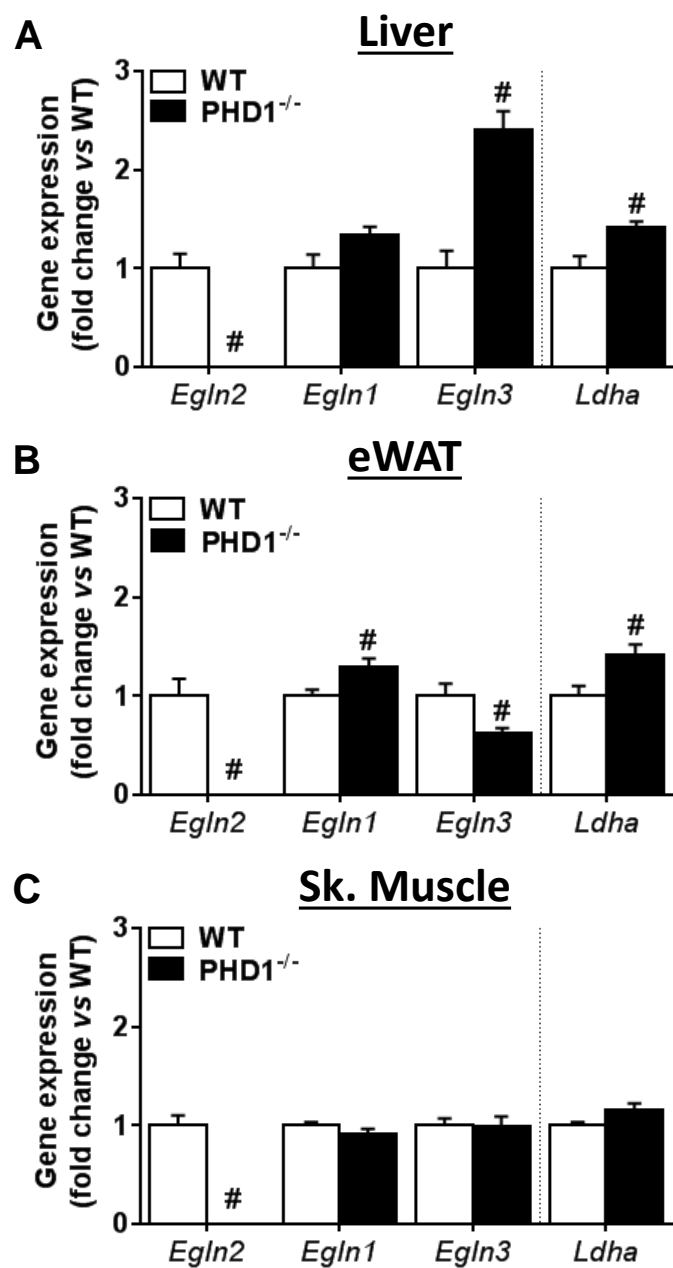
Supplementary Figure 3. Effects of PHD1 deficiency on AMPK signaling pathway in skeletal muscle from low- and high-fat diet-fed mice. Protein expression of AMPK α and ACC, and phosphorylation state of Thr172-AMPK α and Ser79-ACC were assessed by Western blot 15min after insulin injection in skeletal muscle from WT (open bars) and PHD1^{-/-} (black bars) mice on low-fat (LFD) or high-fat (HFD) diet, as described in Figure 3. Representative blots are shown in (A) and densitometric quantifications in (B-G). Phospho/total ratios were calculated and expressed as fold change relative to WT-LFD mice. Data are means \pm SEM (n=4 for LFD-WT; n=7 for LFD-PHD1^{-/-}; n=5 for HFD-WT; n=7 for HFD-PHD1^{-/-}). * p<0.05 vs LFD mice, # p<0.05 vs WT mice

Supplementary Figure 4. Effects of PHD1 deficiency on AMPK signaling and expression of lipogenic proteins and metabolic/inflammatory genes in white adipose tissue from low- and high-fat diet-fed mice. Protein expression of AMPK α , ACC and FAS, and phosphorylation state of Thr172-AMPK α were assessed by Western blot 15min after insulin injection in epididymal white adipose tissue (eWAT) from WT (open bars) and PHD1^{-/-} (black bars) mice on low-fat (LFD) or high-fat (HFD) diet, as described in Figure 3. Representative blots are shown in (A) and densitometric quantifications expressed as fold change relative to WT-LFD mice in (B-F). HSP90 was used for internal housekeeping protein expression. mRNA expression of key genes involved in glucose/FA uptake (*Insr*: Insulin receptor β , *Slc2a4*: GLUT4; *Cd36*: CD36), triglyceride synthesis (*Fasn*: FAS; *Acaca*: ACC1), fatty acid oxidation (*Lipe*: HSL; *Cpt1a*: CPT1 α ; *Ucp1*: UCP1) and adipokines (*Adipoq*: Adiponectin; *Lep*: Leptin) was measured by RT-qPCR (G). mRNA expression of key genes involved in eWAT inflammation (H) was measured by RT-qPCR (*Emr1*: F4/80,

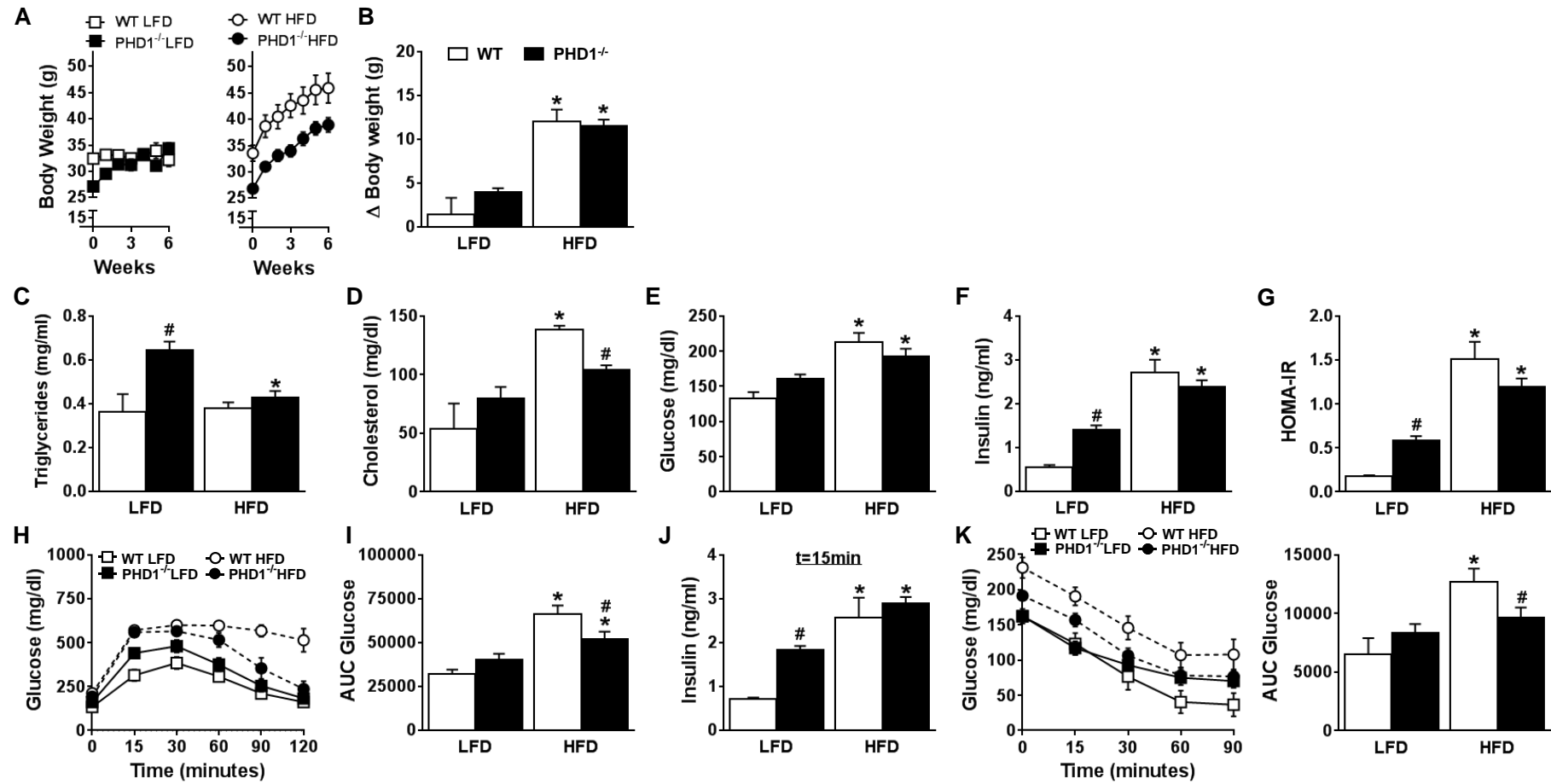
Cd68:CD68; *Arg1*: ARG1; *Itgax*: CD11c; *Ccl2*: MCP-1; *Il1b*: IL1 β ; *Tnfa*: *TNF α*). Results are expressed relative to the housekeeping gene RPLP0 as fold change vs WT-LFD mice. Data are means \pm SEM (n=4 for LFD-WT; n=7 for LFD-PHD1^{-/-}; n=5 for HFD-WT; n=7 for HFD-PHD1^{-/-}). * p<0.05 vs LFD mice, # p<0.05 vs WT mice.

Supplementary Figure 5. PHD1 deficiency increases hepatic lipogenic gene expression in chow-fed mice. Livers from WT (open bars) and PHD^{-/-} (black bars) mice on standard chow diet. The mRNA expression of key genes involved in the regulation of hepatic TG synthesis (A; *Srebf1*: SREBP-1c; *Acaca*: ACC1; *Fasn*: FAS; *Scd1*: SCD1), cholesterol synthesis (A; *Srebf2*: SREBP2 ; *Hmgcr*: HMGCoA reductase; *Hmgcs2*: HMGCoA synthase) and fatty acid oxidation (A; *Ppara*: PPAR α ; *Pdk4*: PDK4 ; *Cpt1a*: CPT-1 α ; *Acox1*: acyl-coA oxidase 1) and glycolysis (B; *Gapdh*, GAPDH; *Eno1*, Enolase; *Pklr*, PK) was measured by RT-qPCR. The results are expressed relative to the housekeeping gene RPLP0 as fold change vs WT mice. Data are means \pm SEM (n=7 for WT; n=13 for PHD1^{-/-}). # p<0.05 vs WT mice.

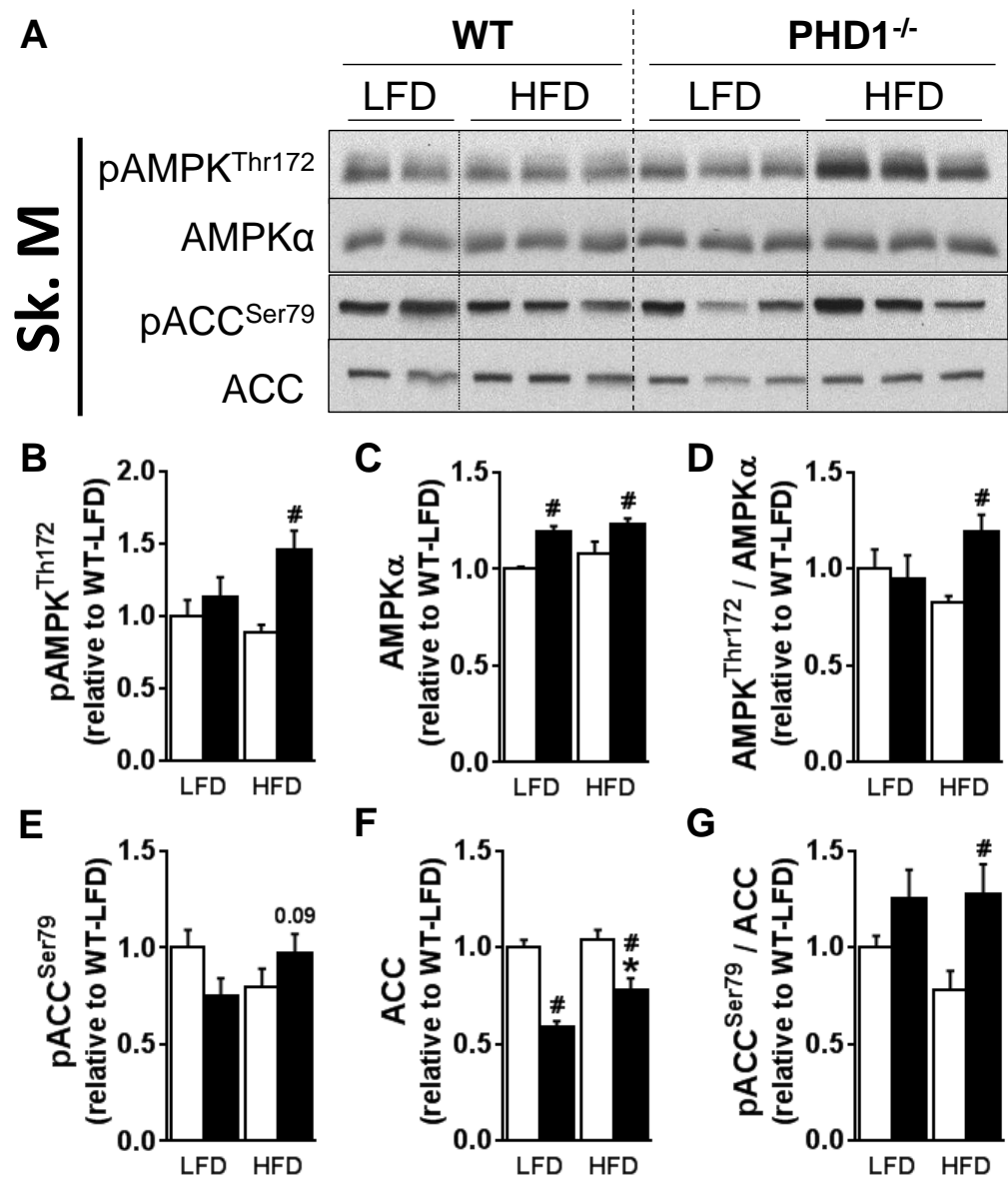
Supplementary Figure 1



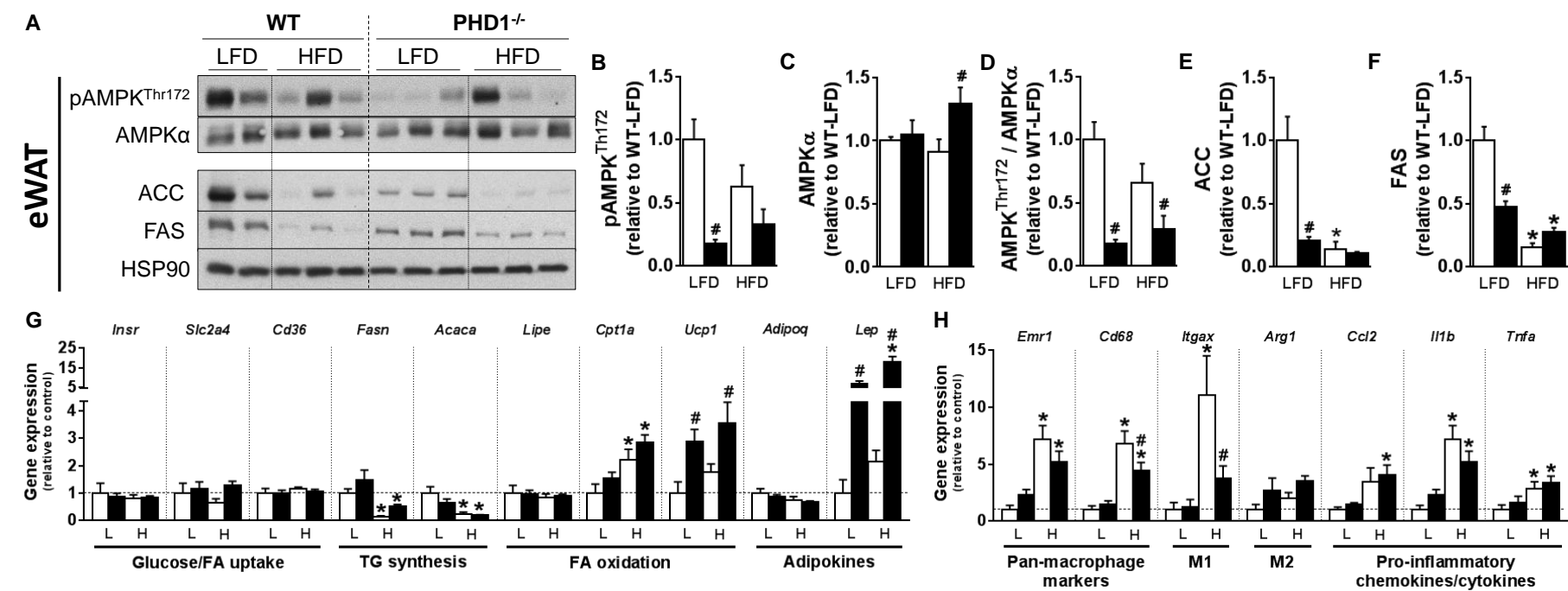
Supplementary Figure 2



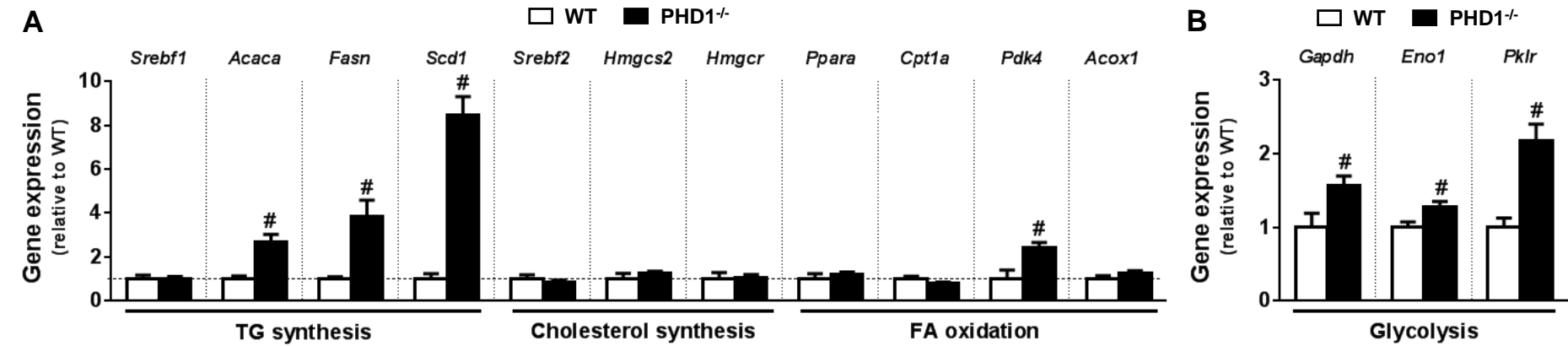
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Table 1: Primary antibodies for Western blots

Primary antibody	Residue	Supplier	Reference	Dilution
ACC	-	Cell Signaling	#3662	1:2000
ACC	Ser79	Cell Signaling	#3661	1:2000
AMPKα	-	Cell Signaling	#2532	1:1000
AMPKα	Thr172	Cell Signaling	#2535	1:1000
FAS		Cell signaling	#3180	1:1000
PKBα+β		Upstate	07-416 + 07-372	1:2000
PKB	Ser473	Cell signaling	#9271	1:1000
HSP90		Santa Cruz	sc-7947	1:1000

Supplementary Table 2: Primer sequences for qRT-PCR

Gene	Accession number	Forward primer	Reverse primer
<i>Acaca</i>	NM_133360.2	CAGCTGGTGCAGAGGTACCG	TCTACTCGCAGGTACTGCCG
<i>Acox1</i>	NM_015729	GGGACCCACAAGCCTCTGCCA	GTGCCGTCAGGCTTCACCTGG
<i>Adipoq</i>	NM_009605	GGAATGACAGGAGCTGAAGG	CGAATGGGTACATTGGGAAC
<i>Arg1</i>	NM_007482.3	GACCACGGGGACCTGGCCTT	ACTGCCAGACTGTGGTCTCCACC
<i>Ccl2</i>	NM_011333.3	TCAGCCAGATGCAGTTAACGCCC	GCTTCTTTGGGACACCTGCTGCT
<i>Cd36</i>	NM_001159558	GCAAAGAACAGCAGCAAAATC	CAGTGAAGGCTCAAAGATGG
<i>Cd68</i>	NM_009853.1	CCTCCACCCTCGCCTAGTC	TTGGGTATAGGATTCGGATTGGA
<i>Cpt1a</i>	NM_013495	AGGAGACAAGAACCCCAACA	AAGGAATGCAGGTCCACATC
<i>Egln1</i>	NM_053207	AGGCTATGTCCGTCACGTTG	TACCTCCACTTACCTTGGCG
<i>Egln2</i>	NM_053208	TCACGTGGACGCAGTAATCC	CGCCATGCACCTTAACATCC
<i>Egln3</i>	NM_028133	AGGCAATGGTGGCTTGCTAT	GACCCCTCCGTGTAACCTTGG
<i>Emr1</i>	NM_010130.4	CTTTGGCTATGGGCTTCCAAGTC	GCAAGGAGGACAGAGTTTATCGTG
<i>Eno1</i>	NM_023119.2	TGGAGAACAAGAAGCACTGG	TGCCAGACCTGTAGAAGTCG
<i>Fasn</i>	NM_007988	CACAGGCATCAATGTCAACC	TTTGGGAAGTCTCAGCAAC
<i>Gapdh</i>	NM_008084.2	TGTGTCCGTCGTGGATCTGA	CCTGCTTCACCACCTTCTTGAT
<i>Hmgcr</i>	NM_008255	CTTGTTGGAATGCCTTGTGATTG	AGCCGAAGCAGCATGAT
<i>Hmgcs2</i>	NM_008256.4	CATCGCAGGAAGTATGCCCCG	GCTGTTTGGGTAGCAGCTCG
<i>Il1b</i>	NM_008361	GACCCCCAAAAGATGAAGGGCT	ATGTGCTGCTGCGAGATTTG
<i>Il6</i>	NM_031168.1	TGTGCAATGGCAATTCTGAT	CTCTGAAGGACTCTGGCTTTG
<i>Insr</i>	NM_010568.2	GCCAAAATTATCATTGGACCCC	CATCCGGCTGCCTCTTTCT
<i>Itgax</i>	NM_021334.2	GCCACCAACCTTCTGGCTG	TTGGACACTCCTGCTGTGCAGTTG
<i>Ldha</i>	NM_010699.2	CCTGTGTGGAGTGGTGTGAA	ATCACCTCGTAGGCACTGTC
<i>Lipe</i>	NM_010719	AGCCTCATGGACCCTCTTCT	GCCTAGTGCCTTCTGGTCTG
<i>Lep</i>	NM_008493	CCCTGTGTGCGTTCTCTGTGGC	GCGGATACCGACTGCGTGTGT
<i>Pdk4</i>	NM_013743	GATTGACATCCTGCCTGACC	CAGGGCTTTCTGGTCTTCTG
<i>Ppara</i>	NM_011144	CAACCCGCCTTTTGTCTATAC	CCTCTGCCTCTTTGTCTTCTG
<i>Pklr</i>	NM_013631	CCTCTGCCTTCTGGATATCGAC	CGATGGTGGCAATGATGCT
<i>Scd1</i>	NM_009127.4	GCTCTACACCTGCCTCTTCGGGAT	TCCAGAGGCGATGAGCCCCG
<i>Slc2a1</i>	NM_011400.3	AGCATCTTCGAGAAGGCAGG	ACAACAAACAGCGACACCAC
<i>Slc2a2</i>	NM_031197.2	TCATGTCGGTGGGACTTGTG	CCCAAGGAAGTCCGCAATGT
<i>Slc2a4</i>	NM_009204	CTCAATGGTTGGGAAGGAAA	GAGGAACCGTCCAAGAATGA
<i>Srebf1</i>	NM_011480	CTGGCTGAGGCGGGATGA	TACGGGCCACAAGAAGTAGA
<i>Srebf2</i>	NM_033218.1	GCGTTCTGGAGACCATGGA	ACAAAGTTGCTCTGAAAACAAATCA
<i>Tnfa</i>	NM_013693	GTCCCCAAAGGGATGAGAAG	CACCTGGTGGTTTGTCTACGA
<i>Ucp1</i>	NM_009463	TCAGGATTGGCCTCTACGAC	TGCATTCTGACCTTCACGAC